Original Research Article

EVALUATION OF REPRODUCTIVE FUNCTIONS IN MALE MICE EXPOSED TO LAMBDA-CYHALOTHRIN AND SOME LOCAL SPICES

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EFFECT OF COADMINISTRATION OF LAMBDA CYHALOTHRIN AND DIFFERENT LOCAL SPICES ON TESTICULAR HISTOLOGY IN SWISS MICE.

Abstract

Aim: This study was aimed at evaluating the effect of coadministration of lambda cyhalothrin and different local spices on testicular histology in Swiss mice

Experimental Design: A completely randomized experimental design using standard methods for analysis.

Location and Duration of Study: This study was carried out in the Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, Rivers State, Nigeria. GPS 4°47'50''N 6°58'49''E The study lasted for 14days.

Methodology: Forty-two male mice were randomly selected into six (6) groups A-F (n=7/group). Group B were gavage 10mg/kg/bw/day of lambda cyhalothrin (LCT) alone. Group C, D, and E were gavaged 10mg/kg/bw/day of LCT and 20mg/kg/bw/day of Tetrepleura tetraptera, Piper guineense, and Xylopia aethiopica respectively, while group F received 10mg/kg/bw/day of LCT and 20mg/kg/bw/day of combination of the three spices. All animals were allowed access to cool clean water and standard rat pellet ad libitum. Twenty-four hours to the termination of the experiment, feed was withdrawn from the animals. From each mouse, 0.5g of testis was fixed in 10% formalin *and sectioned with a Digital Microtome at 5µm thick and stained with Hematoxylin and Eosin (H &E). Photomicrographs were generated with a digital Microscope Biosphere Miller B with an image processor DN2 – Microscopy Image processing Software at X40 magnification.*

Results. Histological analysis of the testes reveal epithelium devoid of spermatogenic element in group B animals administered lambda cyhalothrin only, depicting that LCT impaired spermatogenesis in the mice. In group C the seminiferous epithelium had normal spermatogenic cell complement comparable to the control in group A. In group D, E and F there was gradual regeneration of the interstitial cells of leydig and the accompanying repopulation of the seminiferous epithelium. This reveals that Tetrapleura tetraptera seem to have a greater antioxidant and therapeutic effect against the cyhalothrin-induced reproductive toxicity in exposed animals compared to Piper guineense and Xylopia aethiopica. Therefore, uncontrolled dietary inclusion of Piper guineense and Xylopia aethiopica is not advised as it may hinder spermatogenesis in males. Also, regulatory bodies should ensure that people and wildlife are not exposed to pesticides and insecticides at levels that may cause adverse effects by restricting the handling of such pesticides to Professionals.

Keywords: Histology, pesticides, reproductive toxicity, spermatogenesis, spices,

1.0 INTRODUCTION

Man's survival has been dependent on his curiosity, his desire to examine by trial and error all aspects of his environment and to conclude which materials are medicinal, harmful or of great nutritional value. As such, different plant parts have been used as popular medicine in several countries-underdeveloped, developing and developed-as an alternative treatment for various diseases [1,2]. Several plants, roots, spices and herbs have been reported to be good sources for management against reproductive dysfunctions. Reproductive dysfunction has become a huge concern to researchers due to the fact that a healthy sexual functioning contributes significantly to one's sense of wellbeing and social life. Reports have revealed high incidence of reproductive dysfunction among the population especially the male counterparts.

Some of the factors responsible for infertility are associated with hormonal secretion, erectile impotence, disorders of ejaculation, and toxic effects on the testes and accessory sex organs [3]. To achieve sexual quality, medicinal plants, spices, herbs, plant parts are commonly used for the treatment of male infertility associated disorders- aphrodisiacs [3,4,5] with little or no knowledge about their composition and possible side effect based on the dosage. Medicinal plants are rich sources of antioxidants, cost effective and potent alternative with few and transient side effects in the treatment and management of reproductive disorders, when compared with the existing conventional therapeutic drugs available in the market. It was estimated by [6] that more than two thirds of the world's population relied on plant derived drugs. Local communities have used about ten percent (10%) of all flowering plants on earth to treat various infections, although only one percent (1%) have gained recognition by modern scientists [7]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [8]. These plant-based systems will continue to play an essential role in health care especially in rural areas around the world. Spices generally are parts of various plants cultivated for their aromatic pungent or otherwise desirable substances. They consist of rhizomes, bulbs, flower bud, fruit, seed, and leaves.

Tetrapleura tetraptera is a spice, native to West Africa. It is a gift to human race because of its medicinal properties, used as exotic spice, medicine and as a dietary supplement. *Tetrapleura tetraptera* is traditionally used to treat sicknesses such as diabetes, fibroid, epilepsy, arthritis, asthma, leprosy, convulsion, fertility & sexual issues [9]. The analgesic and anticonvulsant effects of 50-800mg/kg of *Tetrapleura tetraptera* in mice has been demonstrated [10].

Piper guineense (Uziza) is a West African spice plant commonly called Ashanti pepper, African black pepper. The phytochemical studies of the plant revealed the presence of proteins, carbohydrates, alkaloids, steroids, glycosides, saponins, flavonoids, tannins and phenolic compounds. It also contains some vitamins[2,11]. The leaves are used in the treatment of cough, bronchitis, intestinal disease, rheumatism and while the fruits are used as an aphrodisiac and contraction of the uterus [12,13]. Moreover, [14] reported that *Piper guineense* possesses phytochemicals with anti-plasmodial and analgesic potentials.

Many therapeutic effects such as treatment of cough, bronchitis, rheumatism, dysentery and infertility are already being found when using the aerial part of *Xylopia aethiopica* [15]. Previously phytochemical investigation of *Xylopia aethiopica* yielded alkaloids, saponins, tannins, flavonoids and glycosides [15,16], proving its antimicrobial and antibacterial activity [12,15].

Several research studies have indicated that sperm counts have been in decline for decades and scientists say modern lifestyles and contacts with chemicals are a contributing factor. Exposure to pesticides is just one of the reasons for this decline. Pesticides are one of the most potentially harmful chemicals released into the environment without guided regulations. Pyrethroids may be considered safe with low toxicity to mammalian cells but reports have shown different deleterious effects of deltamethrin, [17,18], cypermethrin [19,20] lambda cyhalothrin [21,22] which are endocrine disruptors, by induction of reproductive dysfunction in males[23].

The increasing incidence of male infertility necessitate scientific research into plant and plant parts with fertility enhancing potentials. The available antioxidants are expensive and not readily available to the common man, hence this study was undertaken to evaluate the possible antioxidant capability of *Tetrapleura tetraptera, Piper guineense and Xylopia aethiopica* for reproductive dysfunction in rats exposed to lambda cyhalothrin.

2.0 MATERIALS AND METHODS

2.1 Experimental Location

The study was carried out in the animal house of the Department of Animal & Environmental Biology of the Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Rivers State with GPS coordinate *4^o47'50''N 6^o58'49''E.*

2.2 Experimental animal care and management

Forty-two sexually matured Swiss male mice of mean weight 20.57±3.35g were bought and transported to the Animal house Department of Animal & Environmental Biology, Rivers State University, Port Harcourt Rivers State. They were allowed to acclimatize for 7days before the commencement of the experiment. The rats were fed with standard pellet and clean water *ad libitum*. The experiment was conducted according to the institutional animal care protocols at the Rivers State University, Nigeria and followed approved guidelines for the ethical treatment of experimental animals.

2.3 Test materials

Lambda cyhalothrin was purchased from a chemical store around the study area. The spices were also purchased from a local market around the study area. They were identified and authenticated in the Department of Plant Science and Biotechnology, Rivers State University Port Harcourt by Dr Adeleke MTV, where voucher specimen of dried seeds of the spices are being kept in the herbarium. The selected seeds were dried, crushed into fine powder using a milling machine and stored differently in an air- tight container. The spices were weighed and dosage formulated based on the bodyweight of the mice.

2.3 Experimental design

The experimental animals were separated into 6 groups (A-F) of seven mice each (n=7). Group A was the negative control and so received no test chemical or spices. Group B animals were gavage 10mg/kg/bw/day of lambda cyhalothrin only, Group C animals received 10mg/kg/bw/day of lambda cyhalothrin and 20mg/kg/bw/day of *tetrapleura tetraptera*, Group D received 10mg/kg/bw/day of lambda-cyhalothrin and 20mg/kg/bw/day of *Piper guineense*, Group E received 10mg/kg/bw/day of lambda-cyhalothrin and 20mg/kg/bw/day of *Xylopia aethiopica.* Group F received 10mg/kg/bw/day of lambda-cyhalothrin and 20mg/kg/bw/day of combination of the spices.

Feed was withdrawn from the animals 24hours to the termination of the experiment. Each mouse was dissected and testis removed for histopathological analysis.

2.4 Histopathological evaluation of testis

For each mouse, 0.5g of the testis was fixed in 10% formalin and sectioned with a digital microtome at 5µm thick. Histological sections mounted on slides were stained with Hematoxylin and Eosin (H&E). Photomicrographs were generated with a digital Microscope Biosphere Miller B with an image processor DN2-Microscopy Image Processing Software at x40magnification [18].

3.0 RESULTS

The seminiferous epithelium of the swiss mice exposed to lambda cyhalothrin and some local spices are shown in figure 1-6.

The normal architecture of seminiferous tubules and spermatogenic elements of animals in group A (control) is shown in (figure 1). The seminiferous epithelium is well defined and the interstitial cell of leydig visible. Figure 2 represents the transverse section of testis in group B showing deformed seminiferous epithelium with both mitotic and meiotic elements being degenerated leaving spaces without spermatogenic elements. The interstitial space is empty with loss of interstitial cells of leydig. Figure 3 shows visible regeneration of the interstitial cells of leydig and increase in both mitotic and meiotic spermatogenic elements in group coadministered 10mg/kg/bw/day lambda cyhalothrin and 20 mg/kg/bw/day of *T. tetraptera.* The basal compartment has become lined with regenerated spermatogonia and the interstitial cells of leydig accompanied by spermatogenic element are visible. The seminiferous epithelium of animals in group D exposed to 10mg/kg/bw of cyhalothrin and 20mg/kg/bw/day of *P. guineense,* shows gradual regeneration with fewer distortions in the seminiferous tubules seen when compared to the epithelium of group B animals (Figure 4)**.** Also seen is reduction in the number of interstitial cells of leydig and sertoli cells.

Group E animals exposed to 10mg/kg/bw of lambda cyhalothrin and 20mg/kg/bw/day of *X. aethiopica* is shown in figure 5. The lumen is gradually filled with maturing spermatozoa and some vacuolated sections still visible.

Figure 6 shows sections of seminiferous epithelium in group F with mild signs of reproductive stress including detachment from the basement membrane, leydig cells are not fully regenerated and lumen not tightly filled with maturing spermatozoa.

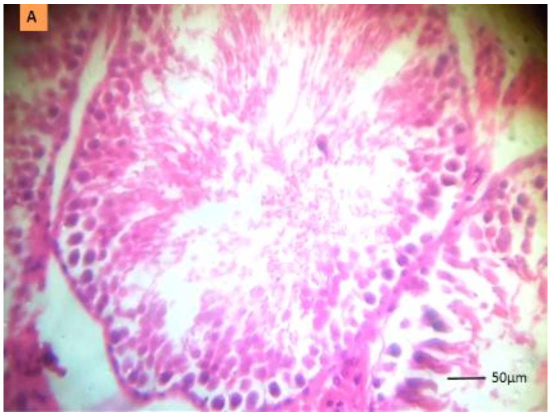

Figure 1:T/S of testis from group A @x40

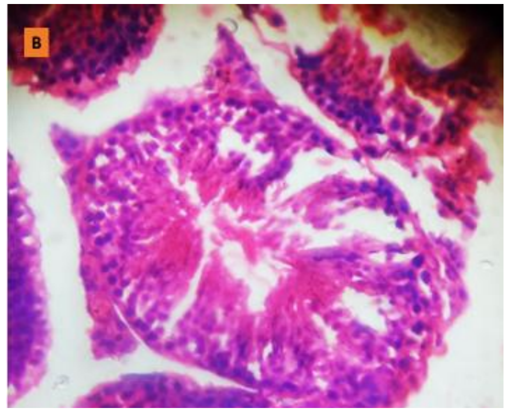

Figure 2: T/S of testis from group B animals @x40

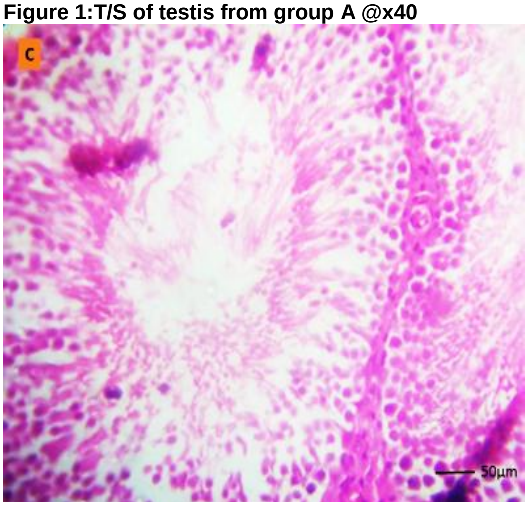

Figure 3: T/S of testis from group C animals @x40

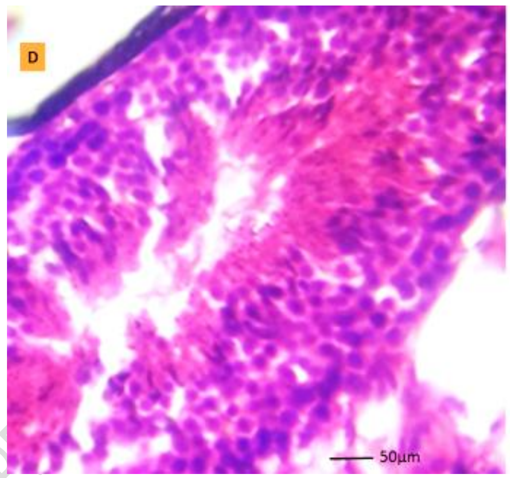

Figure 4: T/S of testis from group D animals @x40

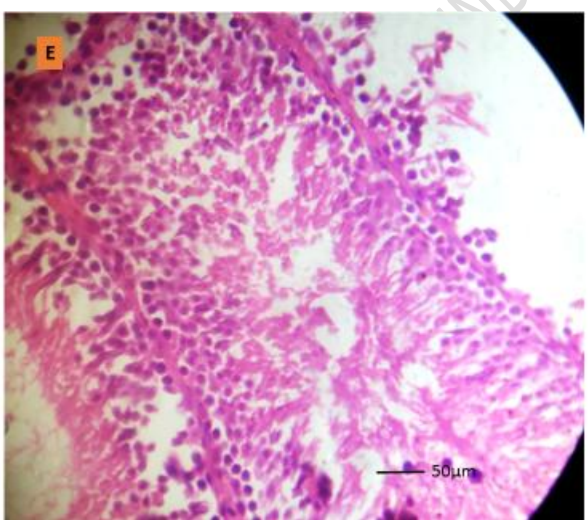

Figure 5: T/S of testis from group E animals @x40

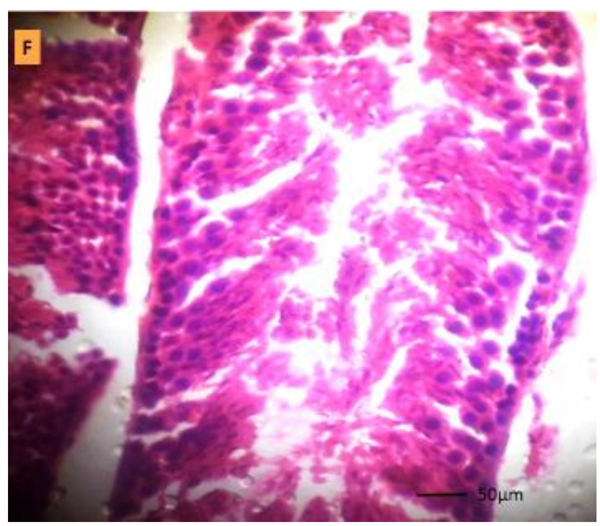

Figure 6: T/S of testis from group F animals @x40

Figure 1 : shows the seminiferous epithelium of the control animals. The epithelium is well defined and the interstitial cell of leydig is visible. Figure 2 shows great degeneration of the seminiferous tubules and spermatogenic element , the

interstitial space is devoid of the interstitial cells of leydig in group B exposed to lambda cyhalothrin only. Figure 3: Photomicrograph of animals in group C coadministered 10mg/kg/bw of LCT and 20mg/kg/bw of *T. tetrapleura.* Tissue shows close resemblance to group A interms of seminiferous epithelium architecture. The interstitial cells of leydig accompanied by spermatogenic element are visible. Figure 4: Photomicrograph of animals in group D coadministered 10mg/kg/bw of LCT and 20mg/kg/bw of *P. guineense*. Tissue still shows fewer distortions in the seminiferous tubules but a reduction in the number of interstitial cells of leydig and sertoli cells. Figure 5: Photomicrograph of animals in group E. The lumen is gradually filled with maturing spermatozoa. The basal compartment is lined with regenerated spermatogonia. Figure 6: Photomicrograph of animals in group E coadministered 10mg/kg/bw of LCT and 20mg/kg/bw of *X. aethiopica*. Tissues show lumen gradually regenerating, but not tighly filled with maturing spermatogonia.

4.0 DISCUSSION

Reproductive dysfunction in males is one of the common causes of social frustration especially in developing countries. Male infertility has been linked to exposure to various environmental toxicant such as pesticides, industrial waste products, steroids, food additives and preservatives. Researchers have shown that pesticides as well as insecticides that exhibit anti-androgenic activity could be responsible for the increased incidence in various male infertility and other sexual disorders. [18,19,20.21, 22,23] .

Evaluation of the transverse section of group B revealed reproductive toxicity biomarkers such as seminiferous tubule degeneration, loss of interstitial cells of leydig that is responsible for the production of androgens such as testosterone. Lambda cyhalothrin inhibited of spermatogonia and meiotic division of primary and secondary spermatocytes as well as the second meiotic division of secondary spermatocytes with spermatids. This is noted as the potential of lambda cyhalothrin to induce reproductive toxicity.

In group C the seminiferous epithelium had normal spermatogenic cell complement comparable to the control in group A. In groups D, E, F there was gradual regeneration of the interstitial cells of leydig and the accompanying repopulation of the seminiferous epithelium. The lumen gradually filled with maturing spermatozoa with some vacuolated sections still visible. This reveals that *T. tetraptera* seem to have a greater antioxidant and therapeutic effect against the Cyhalothrin-induced reproductive toxicity in exposed animals compared to *P. guineense* and *X. aethiopica*.

This is in line with [22] who reported degeneration and loss of spermatogenic elements in mice exposed to lambda cyhalothrin and coadministration of pure extract of *Citrullus lanatus* and *Annona muricata* resulted in regeneration of the spermatogenic elements.

Reduction in the levels of androgens particularly testosterone had been reported on male rats exposed to 40mg/kg/bw/day of cypermethrin by oral administration [24], mice exposed to LCT and different local spices. [23]. Furthermore, histopathological analysis of the testicular epithelium showed congested blood vessels, immature spermatids observed in the adluminal compartment. Also [21] reported oxidative damage of testes and testicular histopathological alterations in rats exposed to LCT while caffeic acid reduced the deleterious effects of lambda cyhalothrin on male fertility. The prevention of cypermethrin-induced reproductive toxicity in rat by resveratrol [24], curcumin and CoQ10 have also been reported [25, 26].

In conclusion, all the spices used in this study possess antioxidant and therapeutic effect against the cyhalothrin -induced reproductive toxicity in exposed animals. However, *Tetrapleura tetraptera* showed greater protective ability compared to *Piper guineense* and *Xylopia aethiopica*. It is hereby recommended that uncontrolled dietary inclusion of *Piper guineense and Xylopia aethiopica is not advised as it may* hinder spermatogenesis in males. Also, regulatory bodies should ensure that people and wildlife are not exposed to pesticides and insecticides at levels that may cause adverse effects by restricting the handling of such pesticides to Professionals.

ETHICAL APPROVAL

The experiment was conducted according to the institutional animal care protocols at the Rivers State University Nkpolu-Oroworukwo, Port Harcourt, Rivers state, Nigeria and followed approved guidelines for the ethical treatment of experimental animals.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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